

# ANTI-DONOR ANTIBODY QUANTITATION FOR PRE- AND POST-TRANSPLANT MONITORING OF A 100% PRA HEART RECIPIENT

R. Siegel, E. Klohe and D. Eklund, Inland Northwest Blood Center, Spokane, WA.

## Introduction

We describe a unique method of analysis for One Lambda FlowPRA™ Single Antigen beads to quantitate specific antibody levels. Combined with innovative plasma-exchange/IVIg protocols and cytolytic B cell therapy, the ability to monitor specific anti-donor antibody was instrumental in allowing successful transplantation of a highly sensitized (100% Class I PRA) heart recipient at risk for humoral rejection. *For details on the clinical case, attend our oral presentation in the Interesting and Difficult Case Studies Workshop, October 29<sup>th</sup> 2:00 –3:30.*

## Materials and Methods

Flow bead assays were performed using FlowPRA™ Class I Single Antigen antibody detection kits (One Lambda, Inc.) and acquired on a FACSCalibur™ flow cytometer (Becton-Dickinson). Analysis was performed utilizing CellQuest™ software (Becton Dickinson) to include region statistics that provided the mean FL1 (FITC) fluorescence for each bead. (**Figure 1**)

Raw fluorescence data from patient and control sera were entered into a spreadsheet developed by Puget Sound Blood Center that contains formulas to subtract the negative control for each bead and correct for non-specific shifts in fluorescence detected on the control bead. This provided a corrected mean fluorescence value for each antigen. On a 1024 channel value scale, corrected shifts > 80 indicated a positive result. (**Figure 2**)

Inhibition of specific anti-HLA antibodies was tracked in the patients' sera following IVIG therapy pre-transplant. This allowed selection of a 6-antigen mismatched donor heart that was predicted to be compatible with post-IVIG sera. However, intra-operative plasma-exchange was performed as an extra precaution, since the transplant was performed prior to the availability of crossmatch results.

## Results

Despite a compatible flow crossmatch, anti-donor antibody appeared within 10 days post-transplant. Although weak C4d and fibrin deposition was noted on biopsy samples, the patient remained stable. Nevertheless, the continuing rise of anti-donor antibody levels prompted us to initiate post-operative plasma-exchange, IVIG and finally cytolytic B cell therapy. Anti-donor antibody levels became undetectable within 2 weeks of cytolytic B cell therapy and have remained so. **Figure 3** shows the patient's anti-donor antibody levels pre-IVIg, post-IVIg at time of transplant, at the peak of anti-donor antibody formation, and current. **Figure 4** shows the patient's anti-donor antibody timeline in the 6 months post-transplant. The timeline also highlights interventions aimed at reducing anti-donor antibody levels and significant biopsy findings.

## Conclusion

This method of flow bead analysis allowed quantitation of antibody against mismatched donor antigens, early detection of potential humoral rejection, correlation with biopsy findings and feedback on the patient's response to immunosuppressive therapies.

### **Acknowledgement**

The authors thank Susan Chloupek at the Puget Sound Blood Center HLA Laboratory for providing the custom fluorescence analysis spreadsheet, and the Inland Northwest Thoracic Organ Transplant Program for providing the clinical and biopsy data.